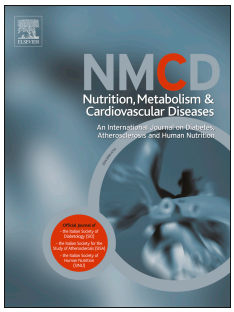
DOI:
10.1016/j.numecd.2016.03.004

Document Version
Peer reviewed version

Accepted Manuscript



Lack of effect of supplementation with EPA or DHA on platelet-monocyte aggregates and vascular function in healthy men. 1-3

Dr S.C. Cottin, A. Alsaleh, T.A.B. Sanders, W.L. Hall

PII: S0939-4753(15)30280-5

DOI: 10.1016/j.numecd.2016.03.004

Reference: NUMECD 1570

To appear in: *Nutrition, Metabolism and Cardiovascular Diseases*

Received Date: 23 October 2015

Revised Date: 7 March 2016

Accepted Date: 8 March 2016

Please cite this article as: Cottin S, Alsaleh A, Sanders T, Hall W, Lack of effect of supplementation with EPA or DHA on platelet-monocyte aggregates and vascular function in healthy men. 1-3, *Nutrition, Metabolism and Cardiovascular Diseases* (2016), doi: 10.1016/j.numecd.2016.03.004.

Lack of effect of supplementation with EPA or DHA on platelet-monocyte aggregates and vascular function in healthy men. 1-3

Cottin SC[a,b]., Alsaleh A[a], Sanders TAB[a], and Hall WL[a].

[a] From King's College London, Diabetes and Nutritional Sciences Division, Faculty of Life Sciences & Medicine, 150 Stamford Street, London SE1 9NH, UK.

[b] Address correspondence to Dr S. C. Cottin, Present address: Micronutrients Group, Lifelong Health

Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB UK. Email: sarah.cottin@abdn.ac.uk. Telephone: +44 (0)1224 438719.

This work was supported by funding from King's College London, Diabetes and Nutritional Sciences Division, Faculty of Life Sciences & Medicine, 150 Stamford Street, London SE1 9NH. Experimental oils provided by Croda Chemicals Europe Ltd, Goole, UK who were not involved in the trial design, data collection, data analysis or reporting of the results.

This trial was registered at clinicaltrials.gov as NCT01735357.

Abstract: 230 words

Text: 3999 words

References: 41

Figures: 1

Tables: 6

On line supplement material: 1

ABSTRACT

(1) Background and Aims: Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in fish oil are postulated to have favourable effects on platelet, endothelial and vascular function. We investigated whether EPA has differential effects on *in vivo* platelet aggregation and other markers of cardiovascular risk compared to DHA.

(2) Methods and Results: Following a 2 wk run-in taking encapsulated refined olive oil, 48 healthy young men were randomly allocated using a parallel design to receive EPA-rich (3.1 g EPA /d) or DHA-rich (2.9 g DHA /d) triglyceride concentrates or refined olive oil (placebo), for a total supplementary lipid intake of 5g/d. The specified primary outcome was change in platelet monocyte aggregates (PMA); secondary outcomes were capillary density, augmentation index, digital pulse volume measurements, 24 h ambulatory BP, plasma 8-isoprostanes-$F_{2\alpha}$. Changes in the proportions of DHA and EPA in erythrocytes and non-esterified fatty acid composition indicated compliance to the intervention. There was no significant treatment effect on PMA (P=0.382); mean changes (%) (95% CI) were placebo -0.5 (-2.0, 1.04), EPA 0.4 (-0.8, 1.6), DHA 0.3 (-1.5, 2.0). R-QUICKI, an index of insulin sensitivity, was greater following EPA compared to placebo (P< 0.05). No other significant differences were noted.

(3) Conclusion: Neither EPA- nor DHA-rich fish oil supplementation influence platelet-monocyte aggregation or several markers of vascular function after 6 wk in healthy young males. This trial was registered at clinicaltrials.gov as NCT01735357.

INTRODUCTION

Epidemiological and observational studies support the theory that fish or fish oil consumption reduces the risk of cardiovascular disease (CVD) [1], while recent meta-analyses found no association between n-3 polyunsaturated fatty acids (n-3 PUFA) supplementation and CVD death, or events [2]. Studies vary in design, populations, duration and doses, as well as the respective ratios of EPA and DHA used, and investigations into their separate effects are limited [3].

Endothelial dysfunction characterizes the initiating stage of atherogenesis, and is closely linked to platelet activation. Nitric oxide produced by the endothelium plays a crucial role in platelet activation, including P-selectin expression and platelet-monocyte aggregation [4]. Platelet-monocyte aggregates (PMA) constitute an early marker of CVD risk [5] and are believed to be a more accurate indicator of true *in vivo* platelet function compared to *ex vivo* measures of platelet aggregation, and also to other *in vivo* measures like platelet surface P-selectin [6, 7]. On the other hand, platelet activation promotes the homing of endothelial progenitor cells (EPCs) to the sites of endothelial injury and favours their differentiation into endothelial cells [8]. The postulated anti-thrombotic actions of fish oils have been mostly attributed to the ability of EPA to compete with arachidonic acid (AA) in the cyclooxygenase (COX) pathway, leading to the formation of eicosanoids that are less pro-thrombotic than the eicosanoids derived from AA [9]. Both EPA and DHA may also give rise to the production of protectins and resolvins that appear to have potent anti-inflammatory actions [9], and may also directly inhibit platelet aggregation [10], which may account for the anti-thrombotic actions of n-3 PUFAs. On the other hand, the additional double bond in DHA may lead to a greater effect on platelet membrane fluidity compared to EPA [11]. EPA and DHA may also enhance endothelial function [12, 13] and improve EPC functionality and numbers in vitro [14] and in healthy subjects at moderate risk of CVD [15], possibly by modulating mobilization, adhesion and angiogenesis mediated by changes in eicosanoid metabolism and/or nitric oxide signalling.

In healthy young men, oily fish consumption (providing ~ 1 g EPA+DHA/d) for 4 wk reduced PMA by 35% [16] and the present study set out to determine whether this effect was primarily influenced by EPA or DHA. It should be noted, however, that the study was designed before the publication of further findings from the same group in 2013, showing that fish oil supplementation (2g/d, 6wks) did not affect PMA in CHD patients [17], nor in cigarette smokers [18]. The overall aim was to determine whether supplementation with oils enriched with either EPA or DHA, had differential effects on markers of platelet, endothelial and vascular function in healthy young males. Our primary outcomes included PMA and numbers of EPC, as a putative marker of endothelial repair [19]. However, EPCs are very rare events and account for 0.0001 - 0.01% of all mononuclear cells. Technical difficulties were encountered in the EPC method, which did not allow the recording of sufficient events to provide accurate results. Thus only the PMA data are reported as primary outcomes. Secondary outcomes included finger capillary density (a marker of microvascular function), plasma 8-isoprostane-$F_{2\alpha}$, arterial stiffness (digital and radial pulse wave analysis), ambulatory blood pressure (BP) / heart rate (HR) measurement, and circulating markers of metabolic dysregulation.

METHODS

Participants

Ethical approval was granted by the Bromley Research Ethics Committee, London in April 2009 (ref. 08/H0805/2) and informed consent was obtained from all participants. Healthy males (aged 18-45 y) were recruited through internal e-mail circulars and posters among King's College London students and staff, and fitness centre users in the vicinity. Exclusion criteria were history of CVD, diabetes (or fasting glucose ≥ 6.1 mmol/L), cancer, kidney, liver or bowel disease; gastrointestinal disorder or use of drug altering nutrient absorption; smoking, history of substance abuse or alcoholism (>60 units/wk); current alcohol intake > 28 units/wk; BP > 160/90 mmHg, fasting blood

cholesterol > 6.5 mmol/L; fasting triacylglycerol concentrations > 2.0 mmol/L, platelet count above or below the normal range or any history indicative of a congenital or acquired platelet or haemostatic defect and recent use of associated medications; allergy or intolerance to study capsules; current consumption of >1 portion oily fish/wk; weight change of >3 kg in preceding 2 months; BMI <18 and >32 kg/m^2. At screening participants filled in a questionnaire on their general health, alcohol and fish consumption; BP, height and weight, body fat percentage, waist and hip circumference were measured, and blood was tested for liver function, fasting glucose, insulin, lipid profile and full blood count.

Intervention

The single-blind, randomized, placebo-controlled, parallel study ran from June 2009 to May 2010. Power calculations were based on 16 subjects per group completing the study to give an 80% power to detect a 10% unit difference in mean PMA at a significance level of 0.05, using standard deviations and expected changes from a previous study [16]. Following a run-in period (5 g refined olive oil/d for 2 wk), participants were randomly assigned to receive either EPA-, DHA-rich oils, or refined olive oil (British Pharmacopoeia specification, maximum unsaponifiable matter 1.5 %) in the form of purified triglycerides in soft gel capsules (Incromega^{TM} EPA500TG SR, DHA500TG SR and refined olive oil capsules were provided by Croda Chemicals Europe Ltd, UK). EPA- and DHA-rich oils were blended with refined olive oil and calculations were made to reach a consumption of ~3 g/d of EPA (3.1 g EPA + 0.10 g DPA + 0.71 g DHA) or DHA (2.9 g DHA + 0.17 g DPA + 0.52 g EPA) for a total lipid intake of 5g/d. During the intervention, participants were asked to avoid medications and consumption of oily fish and dietary supplements. Compliance to treatment was assessed by the incorporation of EPA and DHA into erythrocyte membrane. The composition of NEFA was also assessed as changes in their levels and proportions may impact endothelial and vascular function [20].

Data collection

Participants attended the Metabolic Research Unit at King's College London in the morning at the end of the 2-wk run-in period and of the 6-wk treatment phase. Participants were instructed to consume a low fat evening meal, fast overnight, and avoid drinking alcohol, caffeine and taking part in any strenuous exercise during the day preceding their visit. Ambulatory BP monitors were collected, weight and body composition, seated BP and HR were measured (Omron 705IT). The participant was then asked to rest quietly in the supine position for the vascular measurements (pulse wave analysis - PWA, digital volume pulse - DVP, capillaroscopy), after which a fasting blood sample was collected into evacuated containers (Becton Dickinson) for biochemical analysis.

Measurement of PMA by flow cytometry

Samples were analyzed on an FC500-Beckman Coulter flow cytometer. Blood for PMA analysis was drawn into citrated tubes and kept at room temperature (RT) until analysis within 15 min. The protocol for PMA analysis was adapted from Goodall et al [7]. PMA were identified by the co-expression of CD14 and CD42b (or P-selectin), specifically expressed by monocytes and activated platelets, respectively. Results are expressed as a percentage of total CD14+ events, the gating strategy is described in details in supplement material 1. A blocking mouse immunoglobulin (MOPC31) was used to block Fc binding and non specific binding of monoclonal antibodies to leukocytes. In addition, a CD62P monoclonal antibody blocker was used to block P-selectin binding which may encourage *ex-vivo* binding of platelets to leukocytes. 50 µL of whole blood and 50 µL of HBS buffer were incubated with the appropriate antibody for 30 min at RT, and 250 µl of Optilyse®C solution were added. 250 µl PBS were added after 15 min and the samples were allowed to stand another 5 min at RT prior to the flow cytometry analysis. The acquisition time was 200s and the total events were 25,874 +/- 9,180 and 24,968 +/- 9,024 events for the first and second visit, respectively.

Capillaroscopy

Room temperature was recorded prior to the vascular measurements and remained constant at 24.2 +/- 0.4 °C. Capillary density was measured using the CAM1 Capillary Anemometer and CapiScope Image Acquisition and Analysis software (KK Research Technology Ltd, UK), which allows visualization, capture and storage of video capillaroscopy images onto a computer. Capillaroscopy images were made with participants resting in the supine position. The left hand and forearm were supported at heart level and immobilized using a splint surrounded by cushioning, so that the left anular finger (4^{th} digit) could be placed in a finger clamp underneath the CAM1 system camera. A drop of vegetable oil was applied to the skin measurement area to minimize light reflection. Five images were taken at the dorsum of the middle phalanx, away from the nail fold, where capillaries are perpendicular to the skin, in order to measure functional capillary density as the mean of the capillary counts from each image. The day to day reproducibility of the technique was 4.2% and within-day intra-observer reproducibility was 7.1%.

Vascular measurements, ambulatory blood pressure and heart rate monitoring

Ambulatory BP was monitored over 24h two to three days prior to each visit (TM-2430, A&D Instrument Ltd, U.K.) as previously described [21]. Data were transferred to a computer and analysed using the software provided by the manufacturer. PWA (SphygmoCor Px apparatus with SphygmoCor analysis software, version 7.01, AtCor Medical Pty Limited) and DVP stiffness index and reflection index (DVP-SI and DVP-RI respectively, PulseTrace PCA 2, Micro Medical Ltd) were performed as described earlier [22, 23] in the supine position after 15 min rest. They were carried out in triplicate on the left arm while BP was measured concurrently on the right arm (Omron 705IT).

Plasma isoprostane concentrations, lipid profiles and markers of glucose homeostasis/insulin resistance

8-isoprostane $F_{2\alpha}$ analysis was performed as previously described [22]. Briefly, blood was drawn into citrated tubes, treated with indomethacin (15 µmol/L) and BHT (20 µmol/L) and stored at -80°C until measurement by GC/MS (Agilent Technologies 6890N/5673). Fasting venous blood was collected into evacuated containers: fluoride oxalate tubes for glucose, lithium heparin for insulin and EDTA vacutainers for lipids. Plasma glucose and serum triglycerides, cholesterol, and HDL cholesterol concentrations were measured using standard enzymatic assays. LDL cholesterol was calculated using the Friedwald formula. Apolipoprotein B was measured by a polyethylene glycol enhanced immunoturbidimetric assay (Siemens Healthcare Diagnostics, UK) . Total serum non-esterified FA (NEFA) were measured using an enzymatic colorimetric method assay (NEFA-HR(2) Assay kit, WAKO Chemicals Gmbh, Germany). Insulin was measured by ELISA (Siemens Healthcare Diagnostics Ltd, UK). Insulin sensitivity was assessed by the revised quantitative insulin sensitivity check index (RQUICKI: 1/(log glucose (mg/dL) + logInsulin (µU/mL) + logFFA (mmol/L))). Serum adiponectin analysis was measured using Quantikine ELISA kits (R&D Systems, UK). Inter-assay CV was 6.8% and intra-assay CV was 2.5%.

Erythrocyte and NEFA fatty acid composition

Blood was collected into EDTA vacutainers, erythrocyte lipids and NEFA were extracted as previously described [21]. NEFA were isolated on thin layer chromatography (Partisil®, Whatman international Ltd, Kent, UK) and transesterified with toluene:methanol:acetyl chloride, 20:80:10 (v/v) [24]. The FA methyl esters obtained from erythrocytes and NEFA fractions were separated on an Agilent 6890 Gas Chromatograph (Agilent Technologies UK Ltd, UK) fitted with a flame ionization detector with a 25 m BP75 capillary column [21]. The injection volume was 2 µL, the temperature was 160ºC for 4 min and then rose to 200ºC in 10 min (gradient of 12ºC/min).

Statistical analysis

Data were tested for normality and analysed per protocol using SPSS version 17.0. Data were analysed by analysis of covariance (ANCOVA) of endpoint values, adjusted for ethnicity, age, BMI and baseline value. Normality of the residuals was checked for analysis and log transformation of the data was attempted when required.

RESULTS

Of the 57 individuals assessed for eligibility, 49 were randomized to one of the three treatment groups (**Figure 1**). One participant in the EPA-supplemented group dropped out of the study (lost to follow-up). No adverse effects were reported. The baseline characteristics of the 48 participants that completed the study (16 per group) are described in **Table 1**. Body weight and body composition remained unchanged throughout the 6 wks intervention.

Compliance to treatment

The omega-3 index (proportion of EPA+DHA in erythrocyte) increased by 2.7% weight of total fatty acids (95% CI, +1.5, +4.0, $P = 0.009$) in the DHA-supplemented group and 3.9% (95%CI, +2.7, +5.1, $P < 0.001$) in the EPA-supplemented group. The proportion of EPA in erythrocyte membrane following EPA-rich treatment was greater by 3.0% (95% CI [2.3, 3.8], $P < 0.001$) and DPA *n*-3 (22:5*n*-3) by 1.0% (95% CI [0.6, 1.4], $P < 0.001$) compared to olive oil. Erythrocyte DHA was higher by 1.8% (95% CI [0.5, 3.1], $P = 0.004$) and erythrocyte DPA lower by 0.4% (95% CI [0.0, 0.8], $P = 0.039$) after DHA-rich treatment compared with olive oil. In NEFA, the proportion of EPA ($P < 0.001$), DPA *n*-3 ($P < 0.001$) and DHA ($P = 0.004$) were greater in the EPA-supplemented group, while DHA was higher in the DHA-supplemented group ($P = 0.024$) compared with olive oil. The fatty acid composition of all individual erythrocyte lipids and NEFA are presented in **tables 2** and **3**, respectively.

PMA, vascular function and associated markers

Baseline values did not differ between the groups (ANOVA, adjusted for age, BMI and ethnicity). Supplementation with EPA- and DHA-rich oils had no effect on PMA, functional capillary density, plasma 8-isoprostanes-$F_{2\alpha}$, nor the indices of arterial function measured by PWA and DVP (**Table 4**). Night-time ambulatory HR was reduced by 6.5 bpm (95% CI [-12.2, -0.8], P = 0.021) in the DHA-supplemented group compared to the EPA-supplemented group, while day-time and 24h ambulatory BP and HR remained unchanged by treatment (**table 5)**.

Plasma lipids and insulin sensitivity

There was a statistically significant treatment effect on total NEFA concentrations (P = 0.048) (**Table 6**), although post hoc pairwise comparisons were not statistically significantly different: EPA, -0.12 mmol/L (95% CI [-0.25, 0.00], P = 0.061); DHA, -0.10 mmol/L (95% CI [-0.23, 0.03], P = 0.180) compared to olive oil (Bonferroni post hoc multiple comparison test). RQUICKI was higher by 0.056 (95% CI [0.010, 0.102], P = 0.012) in the EPA-supplemented group compared to olive oil (after Bonferroni adjustment). Glucose, insulin and adiponectin concentrations, TC, triglycerides, LDL, HDL, Apolipoprotein B, triglycerides:HDL, TC/HDL and Apolipoprotein B/LDL ratios did not differ between the groups (Table 6).

DISCUSSION

It is crucial to understand how EPA and/or DHA may affect markers of CVD risk in order to optimise their respective proportions in dietary supplements. The EPA and DHA trial was, to our knowledge, the first human intervention to look at the separate effect of the two marine FA on platelet monocyte aggregation, a true indicator of *in vivo* platelet aggregation and an early marker of CVD risk. Because literature is scarce on this individual effect, other markers of CVD risk were included as secondary outcomes in order to further our understanding of the field. Neither EPA nor DHA had an effect on PMA, vascular function or lipoprotein profiles.

EPA and DHA increased their respective forms in erythrocyte membranes and the plasma NEFA fraction, suggesting good compliance to capsule intake. Erythrocyte DPA, but not DHA, was higher following EPA-rich treatment, confirming elongation beyond DPA is limited. In contrast, both DPA and DHA were greater following EPA supplementation in NEFA, suggesting that conversion from EPA to DHA may occur, as documented before [25]. There is evidence that EPA is preferentially incorporated into phospholipids, cholesterol esters [26] and LDL particles [27], and that DHA appears to be preferentially directed to tissues [26, 28]. However, the effect of fish oil supplementation on fasting NEFA patterns has been poorly studied. Fasting NEFA composition appears to reflect adipose tissue fatty acid profiles, although NEFA and subcutaneous adipose tissue EPA and DHA have been shown to be poorly related following fish oil supplementation [29]. The NEFA fraction may be influenced by the composition of other lipid fractions or tissues and further work in this area may help understand the differential roles of EPA and DHA in cardiovascular health.

Our results contrast with previous placebo-controlled trials showing that both EPA and DHA alone could reduce platelet activation and aggregation [30, 31]. However these studies measured *ex vivo* platelet activation, while the present method is believed to represent platelet activation *in vivo* more accurately [6]. It was previously shown that mackerel consumption (~ 1 g EPA+DHA/d, 4 wks) reduced PMA in healthy young men [16]. The difference in outcome might be due to individual variability, as well as the presence of other components in mackerel, such as selenium or vitamins [32]. Consistent with this, the same authors recently reported that fish oil supplementation (2 g/d, 6 wks) had no effect on PMA and other markers of platelet monocyte activation in CHD patients [17]. Corroborating the results on platelet function, we found that neither EPA nor DHA altered measurements of vascular function. Microvascular function has received little attention with regard to potential effects of n-3 PUFA, but limited previous research suggests DHA, but not EPA, may improve forearm microcirculation [33]. Functional capillary density is an early structural indicator of microvascular abnormalities associated with hypertension [34]. It remained unchanged by EPA

and DHA, perhaps unsurprisingly, in this normotensive subject group. The lack of effect on functional capillary density may be due to the young age and metabolic good health of the population group studied. Indices of arterial stiffness and pulse wave reflection, as measured by DVP and PWA augmentation index, remained unchanged upon EPA and DHA-rich treatment. This is consistent with a recent study in which a 12-month fish oil intervention failed to improve arterial stiffness in an older healthy population [21]. This suggests that daily doses of EPA and DHA (~2-3g), provided together or individually, are not able to improve central and peripheral indices of arterial stiffness/vascular tone, in healthy subjects.

Plasma 8-isoprostane-$F_{2\alpha}$ concentrations remained unchanged following both treatments. Previously, 8-isoprostane-$F_{2\alpha}$ concentrations increased postprandially following an EPA-rich high-fat meal in healthy men, although levels decreased following a DHA-only meal [35, 36], supporting suggestions that EPA and DHA initiate the generation of different oxidized fatty acid metabolites. Chronic intake of EPA and DHA appears to have no effect in healthy men, although it has been observed that fish oils may reduce oxidative stress and lipid peroxidation in populations at higher risk of CVD, and possibly at lower doses [37].

Meta-analyses have convincingly established the TAG lowering effect of fish oils, in both normolipidaemic and hyperlipidaemic subjects [38, 39]. This effect is dose-dependent and greater in individuals with higher baseline concentrations [38]. Fasting plasma TAG concentrationss were low in the present study (mean 0.9 mmol/L), which may explain why there was no TAG lowering effect. The lack of effect on cholesterol levels confirms that fish oils are unlikely to exert a cardioprotective effect through a role in cholesterol metabolism [39, 40]. EPA, but not DHA, improved RQUICKI compared to placebo. This was not supported by changes in adiponectin concentrations, and confirmation with a gold standard measure of insulin sensitivity is required.

A key factor in the lack of effect of treatment might be that participants were healthy (free of cardiovascular disease or other significant cardio-metabolic disorder), and some were recruited in local gymnasiums and therefore may have been particularly health conscious, which would limit the

scope for improvement in CVD risk factors. It should be noted that the baseline omega-3 index was 6.8 % (+/-1.6%) (supplement material 2), approaching the 8% target suggested by Von Schacky in 2004 to reduce the risk of coronary heart disease [41]. Although our intervention improved this index to values above this target (10.5% and 9.3% in the EPA- and DHA-treated group, respectively), it may be advisable to recruit populations with a lower omega-3 index in future trials. The main advantage in investigating cardiovascular health effects in a healthy young group of males is that this approach enables investigation of potential preventative effects, and avoids confounding effects of age or sex. A limitation of the study is that the final sample size of 13-16 per group may have resulted in insufficient statistical power to detect the small changes that would be expected in this population. Furthermore, capsules were supplied in different sizes according to treatment, preventing full double-blinding of treatment allocation. Strengths include the sensitive, validated technical approach to measurement of oxidative stress and platelet function (isoprostanes, PMA), and the randomized design. The PMA measurements represent the true '*in vivo*' platelet function, unlike most techniques previously used that measure *ex vivo* aggregation. The effects of fish oil supplementation on capillary density have rarely been reported. The findings reported here are only relevant to healthy young males, and it seems likely that fish oils do not bring additional health benefits to a population at low cardiovascular risk when provided at 3g/d for 6 wks. However, an additional treatment group receiving combined EPA+DHA was not included in the design, and the possibility that EPA and DHA exert a complementary effect cannot be excluded.

CONCLUSION

In conclusion, an *in vivo* marker of platelet function was not responsive to supplementation with approximately 3g/d of long-chain n-3 PUFA, either in the form of EPA or DHA, compared with olive oil in young men at low risk of CVD. Although the oils were either rich in EPA or DHA, and the study design did not include a combined EPA+DHA treatment, these findings support current views that fish oil supplements have little effect on CVD risk in healthy populations [21,

40], especially if they present a high omega-3 index, low TAG values, or other characteristics indicating a particularly healthy status. While this relatively small study reports mechanistic outcomes rather than validated markers of predicted CVD risk, these findings draw further attention to the importance of defining inclusion criteria and cut off values for healthy populations in fish oil supplementation trials, which may greatly affect the outcome of such interventions.

ACKNOWLEDGMENTS

We are grateful to CRODA Europe Ltd for supplying the oils; and to the participants of the study. We are thankful to Prof Alison Goodall (University of Leicester, UK) for her valuable advice and training on the measurement of PMA, and to Dr Zoe Maniou, for her guidance on the measurement of EPC. The assistance of Roy Sherwood, David Gondi and Robert Gray is gratefully acknowledged. WLH and TABS: conceived and devised the study; SC and AA: organized and conducted the study; SC: prepared the initial manuscript draft; SC, WLH and TABS: edited and revised subsequent drafts; and WLH: had primary responsibility for the final content. All authors read and approved the final manuscript. No conflicts of interest were reported.

328 Reference List

329

330 [1] He K, Song Y, Daviglus ML, Liu K, Van Horn L, Dyer AR, et al. Fish consumption and
331 incidence of stroke: a meta-analysis of cohort studies. Stroke. 2004;35:1538-42.

332 [2] Enns J, Yeganeh A, Zarychanski R, Abou-Setta A, Friesen C, Zahradka P, et al. The impact of
333 omega-3 polyunsaturated fatty acid supplementation on the incidence of cardiovascular events and
334 complications in peripheral arterial disease: a systematic review and meta-analysis. BMC
335 Cardiovascular Disorders. 2014;14:70.

336 [3] Cottin SC, Sanders TA, Hall WL. The differential effects of EPA and DHA on cardiovascular
337 risk factors. Proceedings of the Nutrition Society. 2011;70:215-31.

338 [4] Gkaliagkousi E, Corrigall V, Becker S, de Winter P, Shah A, Zamboulis C, et al. Decreased
339 platelet nitric oxide contributes to increased circulating monocyte-platelet aggregates in
340 hypertension. Eur Heart J. 2009;30:3048-54.

341 [5] Furman MI, Barnard MR, Krueger LA, Fox ML, Shilale EA, Lessard DM, et al. Circulating
342 monocyte-platelet aggregates are an early marker of acute myocardial infarction. Journal of the
343 American College of Cardiology. 2001;38:1002-6.

344 [6] Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating Monocyte-
345 Platelet Aggregates Are a More Sensitive Marker of In Vivo Platelet Activation Than Platelet
346 Surface P-Selectin. Circulation. 2001;104:1533-7.

347 [7] Goodall AH, Appleby J. Flow-cytometric analysis of platelet-membrane glycoprotein
348 expression and platelet activation. Methods Mol Biol. 2004;272:225-53.

349 [8] Langer HF, May AE, Vestweber D, De Boer HC, Hatzopoulos AK, Gawaz M. Platelet-induced
350 differentiation of endothelial progenitor cells. Seminars in thrombosis and hemostasis.
351 2007;33:136-43.

352 [9] Calder PC. Mechanisms of Action of (n-3) Fatty Acids. The Journal of Nutrition.
353 2012;142:592S-9S.

354 [10] Lagarde M, Liu M, Véricel E, Calzada C, Chen P, Driss F, et al. Docosahexaenoic acid,
355 protectin synthesis: relevance against atherothrombogenesis. Proceedings of the Nutrition Society.
356 2014;73:186-9.

357 [11] Hashimoto M, Hossain S, Yamasaki H, Yazawa K, Masumura S. Effects of eicosapentaenoic
358 acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. Lipids.
359 1999;34:1297-304.

360 [12] Li Q, Zhang Q, Wang M, Liu F, Zhao S, Ma J, et al. Docosahexaenoic acid affects endothelial
361 nitric oxide synthase in caveolae. ArchBiochemBiophys. 2007;466:250-9.

362 [13] Li Q, Zhang Q, Wang M, Zhao S, Ma J, Luo N, et al. Eicosapentaenoic acid modifies lipid
363 composition in caveolae and induces translocation of endothelial nitric oxide synthase. Biochimie.
364 2007;89:169-77.

365 [14] Devaraj S, Chien A, Rao B, Chen X, Jialal I. Modulation of endothelial progenitor cell number
366 and function with n-3 polyunsaturated fatty acids. Atherosclerosis. 2013;228:94-7.

367 [15] Wu S-Y, Mayneris-Perxachs J, Lovegrove JA, Todd S, Yaqoob P. Fish-oil supplementation
368 alters numbers of circulating endothelial progenitor cells and microparticles independent of eNOS
369 genotype. (In press). The American Journal of Clinical Nutrition 2014;100:1-12.

[16] Din JN, Harding SA, Valerio CJ, Sarma J, Lyall K, Riemersma RA, et al. Dietary intervention with oil rich fish reduces platelet-monocyte aggregation in man. Atherosclerosis. 2008;197:290-6.

[17] Din JN, Sarma J, Harding SA, Lyall K, Newby DE, Flapan AD. Effect of ω-3 fatty acid supplementation on endothelial function, endogenous fibrinolysis and platelet activation in patients with a previous myocardial infarction: a randomised controlled trial. BMJ Open. 2013;33:e003054.

[18] Din JN, Archer RM, Harding SA, Sarma J, Lyall K, Flapan AD, et al. Effect of ω-3 fatty acid supplementation on endothelial function, endogenous fibrinolysis and platelet activation in male cigarette smokers. Heart. 2013;99:168-74.

[19] Sen S, McDonald SP, Coates PTH, Bonder CS. Endothelial progenitor cells: novel biomarker and promising cell therapy for cardiovascular disease. Clinical Science. 2010;120:263-83.

[20] Newens KJ, Thompson AK, Jackson KG, Wright J, Williams CM. Acute effects of elevated NEFA on vascular function: a comparison of SFA and MUFA. Br J Nutr. 2011;105:1343-51.

[21] Sanders TA, Hall WL, Maniou Z, Lewis F, Seed PT, Chowienczyk PJ. Effect of low doses of long-chain n−3 PUFAs on endothelial function and arterial stiffness: a randomized controlled trial. The American Journal of Clinical Nutrition. 2011;94:973-80.

[22] Hall WL, Sanders KA, Sanders TA, Chowienczyk PJ. A high-fat meal enriched with eicosapentaenoic acid reduces postprandial arterial stiffness measured by digital volume pulse analysis in healthy men. J Nutr. 2008;138:287-91.

[23] Hall WL, Formanuik NL, Harnpanich D, Cheung M, Talbot D, Chowienczyk PJ, et al. A Meal Enriched with Soy Isoflavones Increases Nitric Oxide-Mediated Vasodilation in Healthy Postmenopausal Women. The Journal of Nutrition. 2008;138:1288-92.

[24] Lepage G, Roy CC. Specific methylation of plasma nonesterified fatty acids in a one-step reaction. Journal of Lipid Research. 1988;29:227-35.

[25] Egert S, Kannenberg F, Somoza V, Erbersdobler HF, Wahrburg U. Dietary alpha-linolenic acid, EPA, and DHA have differential effects on LDL fatty acid composition but similar effects on serum lipid profiles in normolipidemic humans. JNutr. 2009;139:861-8.

[26] Hansen J-B, Grimsgaard S, Nilsen H, Nordøy A, Bønaa K. Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on fatty acid absorption, incorporation into serum phospholipids and postprandial triglyceridemia. Lipids. 1998;33:131-8.

[27] Egert S, Somoza V, Kannenberg F, Fobker M, Krome K, Erbersdobler HF, et al. Influence of three rapeseed oil-rich diets, fortified with alpha-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid on the composition and oxidizability of low-density lipoproteins: results of a controlled study in healthy volunteers. EurJClinNutr. 2007;61:314-25.

[28] Egert S, Lindenmeier M, Harnack K, Krome K, Erbersdobler HF, Wahrburg U, et al. Margarines Fortified with α-Linolenic Acid, Eicosapentaenoic Acid, or Docosahexaenoic Acid Alter the Fatty Acid Composition of Erythrocytes but Do Not Affect the Antioxidant Status of Healthy Adults. The Journal of Nutrition. 2012;142:1638-44.

[29] Walker CG, Browning LM, Stecher L, West AL, Madden J, Jebb SA, et al. Fatty acid profile of plasma NEFA does not reflect adipose tissue fatty acid profile. British Journal of Nutrition. 2015;114:756-62.

[30] Woodman RJ, Mori TA, Burke V, Puddey IB, Barden A, Watts GF, et al. Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients. Atherosclerosis. 2003;166:85-93.

[31] Phang M, Lincz LF, Garg ML. Eicosapentaenoic and Docosahexaenoic Acid Supplementation Reduces Platelet Aggregation and Hemostatic Markers Differentially in Men and Women. The Journal of Nutrition. 2013;143:457-63.

[32] Violi F, Pignatelli P, Basili S. Nutrition, Supplements, and Vitamins in Platelet Function and Bleeding. Circulation. 2010;121:1033-44.

[33] Mori TA, Watts GF, Burke V, Hilme E, Puddey IB, Beilin LJ. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. Circulation. 2000;102:1264-9.

[34] Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Structural skin capillary rarefaction in essential hypertension. Hypertension. 1999;33:998-1001.

[35] Rontoyanni VG, Hall WL, Pombo-Rodrigues S, Appleton A, Chung R, Sanders TAB. A comparison of the changes in cardiac output and systemic vascular resistance during exercise following high-fat meals containing DHA or EPA. British Journal of Nutrition. 2012;108:492-9.

[36] Purcell R, Latham SH, Botham KM, Hall WL, Wheeler-Jones CP. High-fat meals rich in EPA plus DHA compared with DHA only have differential effects on postprandial lipemia and plasma 8-isoprostane F2α concentrations relative to a control high–oleic acid meal: a randomized controlled trial. The American Journal of Clinical Nutrition. 2014;100:1019-28.

[37] Giordano E, Visioli F. Long-chain omega 3 fatty acids: Molecular bases of potential antioxidant actions. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA). 2014;90:1-4.

[38] Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis. 2006;189:19-30.

[39] Eslick GD, Howe PR, Smith C, Priest R, Bensoussan A. Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. Int J Cardiol. 2009;136:4-16.

[40] Hallund J, Madsen BO, Bugel SH, Jacobsen C, Jakobsen J, Krarup H, et al. The effect of farmed trout on cardiovascular risk markers in healthy men. Br J Nutr. 2010;104:1528-36.

[41] Harris WS, von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? Preventive Medicine. 2004;39:212-20.

TABLES

Table 1: Baseline characteristics of the participants

		Placebo (n=16)	EPA (n=16)	DHA (n=16)	*P* value
Age (y)		25.8 (7.4)	25.8 (7.5)	27.9 (8.0)	0.663[2]
BMI (kg/m^2)		23.6 (3.4)	22.9 (3.0)	23.3 (2.9)	0.805[2]
Waist circumference (cm)		81.7 (10.2)	78.4 (7.4)	82.5 (10.3)	0.459[2]
Systolic BP (mmHg)		119.5 (10.4)	119.5 (13.5)	124.2 (10.7)	0.428[2]
Diastolic BP (mmHg)		66.7 (6.3)	67.9 (11.8)	70.9 (8.7)	0.420[2]
Fasting plasma glucose (mmol/L)		4.9 (0.3)	5.1 (0.3)	5.3 (0.4) [a]	**0.010**[2]
Fasting triglycerides[1] (mmol/L)		0.8 (0.3)	0.9 (0.4)	0.8 (0.3)	0.420[2]
TC:HDL-C ratio		3.2 (0.7)	3.4 (0.8)	3.5 (0.8)	0.546[2]
Ethnicity	White/Caucasian	9	12	9	0.904[3]
	Black	2	1	2	
	Indian/Middle East	4	2	3	
	Asian	1	1	2	

Mean values ± SD.; TC: total cholesterol, HDL-C: high density lipoprotein cholesterol

[1] geometric means.

[2] *P* values are from univariate analysis of the variances of the screening values by treatment

[3] *P* value is from Pearson's Chi-square test of ethnicity by treatment.

[a] P < 0.05 compared to placebo and DHA-supplemented groups, Bonferroni's multiple comparison test

Table 2: Erythrocyte fatty acid composition (%) at baseline and after 6 wks of supplementation in healthy men with olive oil (placebo), EPA- or DHA-rich oils (3g/d).

	Baseline (*n*=46)	Placebo (*n*=15)	EPA (*n*=16)	DHA (*n*=15)	*P* value
16:0 (PA)	17.0 (2.8)	17.5 (3.1)	18.4 (1.7)	17.0 (3.1)	0.226
16:1n-7	0.3 (0.1)	0.5 (0.5)	0.3 (0.1)	0.3 (0.1)	0.91
18:0 (SA)	15.3 (1.5)	15.3 (1.1)	15.7 (1.3)	15.3 (1.9)	0.44
18:1 trans	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.675
18:1n-9	14.9 (1.2)	14.8 (1.1)	14.6 (1.3)	15.3 (1.4)	0.142
18:1n-7	1.2 (0.2)	1.3 (0.1)	1.2 (0.1)	1.2 (0.2)	0.128
18:2n-6 (LA)	12.1 (1.1)	12.2 (1.6)[a]	10.4 (1.7)[b]	11.3 (1.3)[a,b]	0.003
18:3n-3 (ALA)	0.2 (0.1)	0.2 (0.04)	0.17 (0.1)	0.2 (0.04)	0.144
20:3n-6 (DGLA)	2.2 (0.6)	2.3 (1.0)[a]	1.7 (0.4)[b]	2.0 (0.7)[a,b]	0.01
20:4n-6 (AA)	17.3 (2.2)	17.1 (2.2)[a]	15.1 (1.9)[b]	16.9 (2.9)[a]	< 0.001
20:5n-3 (EPA)	1.1 (0.4)	1.1 (0.4)[a]	4.2 (1.4)[b]	1.6 (0.6)[a]	< 0.001
22:4n-6	3.6 (0.9)	3.6 (0.8)[a]	2.7 (0.9)[b]	3.4 (1.1)[a]	0.003
22:5n-6	0.6 (0.1)	0.6 (0.1)[a]	0.4 (0.1)[b]	0.6 (0.1)[a]	< 0.001
22:5n-3 (DPA)	3.3 (0.6)	3.3 (0.6)[a]	4.3 (0.4)[b]	3.1 (0.6)[a]	< 0.001

22:6n-3 (DHA)	5.7 (1.53)	6.0 (1.3)[a]	6.3 (1.2)[a,b]	7.7 (2.0)[b]	0.025
Omega-3 index (EPA+DHA)	6.8 (1.6)	7.1 (1.4)[a]	10.5 (2.4)[b]	9.3 (2.4)[b]	< 0.001

Values are geometric means (SD) , expressed in percentage weight of total fatty acids. PA: palmitic acid, SA: stearic acid, LA: linoleic acid, ALA: alpha-linolenic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, EPA: Eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid.

P value is from univariate analysis of variance of the follow-up value by treatment with baseline value, ethnicity, BMI and age as covariates. Values in the same row with different superscripts are significantly different from each other $P<0.05$ using Bonferroni's test for 3 comparisons.

Table 3: NEFA composition (%) at baseline and after 6 wks of supplementation in healthy men with olive oil (placebo), EPA- or DHA-rich oils (3g/d).

	Baseline (n=46)	Placebo (n=16)	EPA (n=16)	DHA (n=16)	*P* value
16:0 (PA)	41.7 (6.5)	37.6 (6.8)	38.5 (8.0)	39.9 (7.3)	0.630
16:1n-7	2.3 (1.0)	2.2 (0.8)	2.6 (1.0)	2.2 (0.9)	0.889
18:0 (SA)	15.2 (5.0)	16.7 (3.5)	15.8 (5.0)	16.6 (4.6)	0.898
18:1n-9	15.4 (9.4)	20.2 (8.0)[a]	14.3 (7.7)[b]	15.9 (8.4)[a,b]	0.035
18:2n-6 (LA)	4.9 (4.0)	7.2 (3.4)	5.6 (4.2)	4.6 (5.2)	0.655
18:3n-3 (ALA)	1.7 (0.7)	1.5 (0.6)	1.8 (0.7)	1.6 (0.6)	0.066
18:3n-6 (GLA)	0.0 (0.2)	0.0 (0.2)	0.0 (0.2)	0.0 (0.2)	0.409
20:3n-6 (DGLA)	0.6 (0.4)	0.6 (0.2)	0.5 (0.4)	0.8 (0.3)	0.280
20:4n-6 (AA)	1.5 (0.9)	1.4 (0.4)	1.8 (1.2)	1.9 (1.0)	0.077
20:5n-3 (EPA)	0.0 (0.2)	0.4 (0.9)[a]	1.8 (2.9)[b]	0.5 (0.5)[a]	<0.001
22:4n-6	2.8 (2.1)	3.1 (3.0)	3.1 (3.0)	3.1 (3.7)	0.328
22:5n-6	0.0 (0.3)	0.3 (0.1)	0.3 (0.4)	0.3 (0.2)	0.086
22:5n-3 (DPA)	0.0 (0.2)	0.0 (0.3)[a]	0.6 (0.7)[b]	0.0 (0.3)[a]	0.004
22:6n-3 (DHA)	0.8 (0.3)	0.8 (0.7)[a]	1.7 (1.8)[b]	1.5 (1.6)[b]	<0.001

Values are geometric means (SD), expressed in mass percentage of total NEFA composition. PA: palmitic acid, SA: stearic acid, LA: linoleic acid, ALA: alpha-linolenic acid, GLA: gamma-linolenic acid, DGLA: dihomo-gamma-linolenic acid, AA: arachidonic acid, EPA: Eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid.

P value is from univariate analysis of variance of the endpoint values by treatment with baseline values, ethnicity, BMI and age as covariates. Values in the same row with different superscripts are significantly different from each other, $P<0.05$ using Bonferroni's test for 3 comparisons.

Table 4: Platelet mononuclear cell aggregates, capillary density, plasma 8-isoprostanes-F2α, and parameters of vascular function at baseline and changes after 6 wks of supplementation in healthy men with olive oil (placebo), EPA- or DHA-rich oils (3g/d).

		Olive Oil (n = 15)	EPA (n = 15)	DHA (n = 15)	*P* value[4]
PMA (CD14+/CD42b+) (% total CD14+)	Baseline	9.6 ± 1.8	7.9 ± 2.6	8.2 ± 2.2	
	Change	-0.5 (-2.0, 1.04)[1]	0.4 (-0.8, 1.6)[2]	0.3 (-1.5, 2.0)[1]	0.917
Capillary density count (capillaries/mm^2)	Baseline	65.0 ± 8.6	68.9 ± 12.0	69.4 ± 6.5	
	Change	4.0 (-1.7, 9.7)	2.0 (-1.8, 5.8)	0.4 (-6.3 , 7.0)[3]	0.773
Plasma 8-isoprostanes (ng/L)	Baseline	75.2 ± 36.1	88.1 ± 28.3	76.5 ± 30.4	
	Change	3.3 (-5.6, 12.3)	1.0 (-13.7, 15.7)	-7.2 (-24.0 , 9.6)[1]	0.261
Stiffness index, *m/s*	Baseline	5.7 (0.4)	6.12 (0.8)	6.1 (1.6)	0.706
	Change	-0.0 (-0.5 , 0.4)[1]	-0.2 (-0.6 , 0.1)	-0.0 (-0.2 , 0.1)	
Reflection index, *%*	Baseline	65.4 (11.9)	68.0 (11.3)	64.6 (11.7)	0.797
	Change	0.8 (-8.8 , 10.3)[1]	-1.6 (-6.5 , 3.4)	0.2 (-6.7 , 7.0)	
Peripheral AIx, *%*	Baseline	54.0 (9.0)	53.7 (13.2)	50.0 (15.1)	0.645
	Change	-2. 5 (-6.6 , 1.7)[1]	-3.5 (-8.5 , 1.5)	0.3 (-3. 7 , 4.3)	
Central AIx, *%*	Baseline	112.2 (8.3)	110. 6 (11.3)	108.4 (11.0)	0.560
	Change	-2.1 (-6.9 , 2.7)[1]	-2.9 (-8.7 , 2.8)	-2.6 (-6.1 , 0.9)	

Mean ± SD or mean change (95% CI); PMA: platelet monocyte aggregates, SBP: systolic blood pressure, DBP, diastolic blood pressure, HR: heart rate, AIx: Augmentation index

PMA are expressed as percentage of total gated monocytes; AIx = P2/P1, where P1 and P2 are the pressure of the forward (systolic) and reflected (diastolic) wave, respectively.

[1] n=13, [2] n=14 , [3] n=12,

[4] P values are from univariate analysis of variances of the endpoint values by treatment with baseline values, ethnicity, BMI and age as covariates.

Table 5: Ambulatory blood pressure and heart rate at baseline and after 6 wks of supplementation in healthy men with olive oil (placebo), EPA- or DHA-rich oils (3g/d).

		Olive Oil (*n* = 15)	EPA (*n* = 14)	DHA (*n* = 15)	*P* value
Day					
SBP (mmHg)	Baseline	122.8 (10.5)	124.9 (8.6)	125.2 (7.7)	
	Change	-0.5 (-4.2 ; 3.3)	-1.9 (-6.1 ; 2.2)	-1.9 (-6.1 ; 2.2)	0.859
DBP (mmHg)	Baseline	72.0 (6.8)	75.1 (5.3)	72.3 (7.8)	
	Change	0.3 (-2.9 ; 3.5)	-1.8 (-4.2 ; 0.6)	-1.0 (-3.7 ; 1.7)	0.643
HR (bpm)	Baseline	65.9 (7.7)	70.2 (10.9)	67.1 (9.0)	
	Change	1.8 (-2.1 ; 5.8)	0.1 (-5.9 ; 6.2)	-0.5 (-3.8 ; 2.7)	0.580
Night					
SBP (mmHg)	Baseline	101.1 (8.3)	107.2 (10.8)	103.7 (8.0)	
	Change	0.9 (-3.8 ; 5.6)	0.7 (-4.5 ; 5.9)	1.4 (-3.7 ; 6.5)	0.246
DBP (mmHg)	Baseline	55.4 (4.7)	61.1 (3.8)	58.7 (6.4)	
	Change	3.3 (-0.5 ; 7.0)	1.8 (-0.9 ; 4.5)	0.5 (-3.1 ; 4.1)	0.392
HR (bpm)	Baseline	54.8 (5.4)	56.9 (7.0)	56.5 (8.0)	
	Change	-0.2 (-5.3 ; 4.9)[a,b]	3.0 (-1.2 ; 7.2)[a]	-2.7 (-5.4 ; 0.1)[b]	**0.021**

24h

SBP (mmHg)	Baseline	117.4 ± 9.5	121.0 ± 8.5	120.5 ± 7.1	
	Change	-1.4 (-5.3, 2.6)	-1.6 (-5.3, 2.0)[2]	-2.1 (-5.6, 1.5)	0.663
DBP (mmHg)	Baseline	70.3 ± 10.7	72.1 ± 4.8	69.3± 6.9	
	Change	-2.4 (-8.6, 3.9)	-1.1 (-3.4 , 1.1)[2]	-1.3 (-3.2, 0.7)	0.061
HR (bpm)	Baseline	62.5 (6.7)	67.4 (9.9)	64.5 (8.5)	
	Change	0.9 (-3.2 ; 5.0)	0.5 (-4.9, 5.9)[2]	-1.3 (-3.9 ; 1.2)	0.186

Data are presented as means (SD) and changes from baseline are expressed as mean of the differences (CI 95%).

P values are from univariate analysis of variance of the endpoint values by treatment with baseline values, ethnicity, BMI and age as covariates.

Values in the same row with different superscripts are significantly different from each other, $P<0.05$ using Bonferroni's test for 3 comparisons.

Table 6: Plasma lipid concentrations and indices of glycaemic control and insulin sensitivity at baseline and after 6 wks of supplementation in healthy men with Olive Oil (placebo), EPA or DHA (3g/d).

		Olive Oil (n=16)	EPA (n=16)	DHA (n=16)	*P* value[2]
Total cholesterol	Baseline	4.75 ± 0.72	4.69 ± 0.97	4.45 ± 0.67	
(mmol/L)	Change	-0.10 (-0.46, 0.26)	0.00 (-0.38, 0.38)	0.18 (-0.15, 0.51)	0.673
Triglycerides (mmol/L)	Baseline	0.88 ± 0.31	0.97 ± 0.45	0.87 ± 0.43	
	Change	-0.11 (-0.26, 0.03)	-0.26 (-0.46, -0.07)	-0.12 (-0.30, 0.07)	0.396
HDL-C[1] (mmol/L)	Baseline	1.49 ± 0.36	1.45 ± 0.28	1.37 ± 0.29	
	Change	0.02 (-0.17, 0.21)	0.16 (-0.04, 0.35)	0.06 (-0.03, 0.15)	0.680
LDL-C (mmol/L)	Baseline	2.87 ± 0.57	2.81 ± 0.76	2.68 ± 0.54	
	Change	-0.07 (-0.34, 0.20)	-0.04 (-0.28, 0.19)	0.18 (-0.05, 0.41)	0.404
NEFA (mmol/L)	Baseline	0.32 ± 0.09	0.38 ± 0.16	0.37 ± 0.24	
	Change	0.05 (-0.04, 0.14)	-0.09 (-0.20, 0.01)	-0.08 (-0.19, 0.03)	0.048
TC:HDL-C ratio	Baseline	3.34 ± 0.80	3.31 ± 0.64	3.38 ± 0.80	
	Change	-0.13 (-0.38, 0.13)	-0.24 (-0.42, -0.06)	-0.06 (-0.25, 0.13)	0.367
Apolipoprotein B (g/L)	Baseline	0.88 ± 0.19	0.90 ± 0.23	0.80 ± 0.25	
	Change	-0.07 (-0.19, 0.05)	-0.06 (-0.11, -0.01)	0.08 (-0.05, 0.21)	0.264

Glucose (mmol/L)	Baseline	5.0 ± 0.3	5.2 ± 0.4	5.3 ± 0.3	
	Change	0.1 (-0.2, 0.4)	-0.0 (-0.4, 0.3)	-0.0 (-0.2, 0.2)	0.935
Insulin[1] (mU/L)	Baseline	5.4 (3.1)	5.6 ± 2.2	6.4 ± 5.1	
	Change	1.4 (-0.3, 3.1)	0.8 (-1.3, 3.0)	0.3 (-1.3, 1.9)	0.826
Adiponectin (μg/L)	Baseline	6.7 ± 2.8	7.2 ± 3.6	7.5 ± 3.5	
	Change	1.4 (0.1, 2.8)	0.7 (-0.9, 2.2)	0.0 (-1.0, 1.0)	0.281
RQUICKI	Baseline	0.47 ± 0.06	0.45 ± 0.04	0.45 ± 0.08	
	Change	-0.03 (-0.07, 0.01)	0.04 (-0.01, 0.08)[a]	0.00 (-0.03, 0.03)	0.014

Mean ± SD or mean change (95% CI); HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol (LDL-C); NEFA, non-esterified fatty acids; TC, total cholesterol; RQUICKI, revised quantitative insulin sensitivity check index.

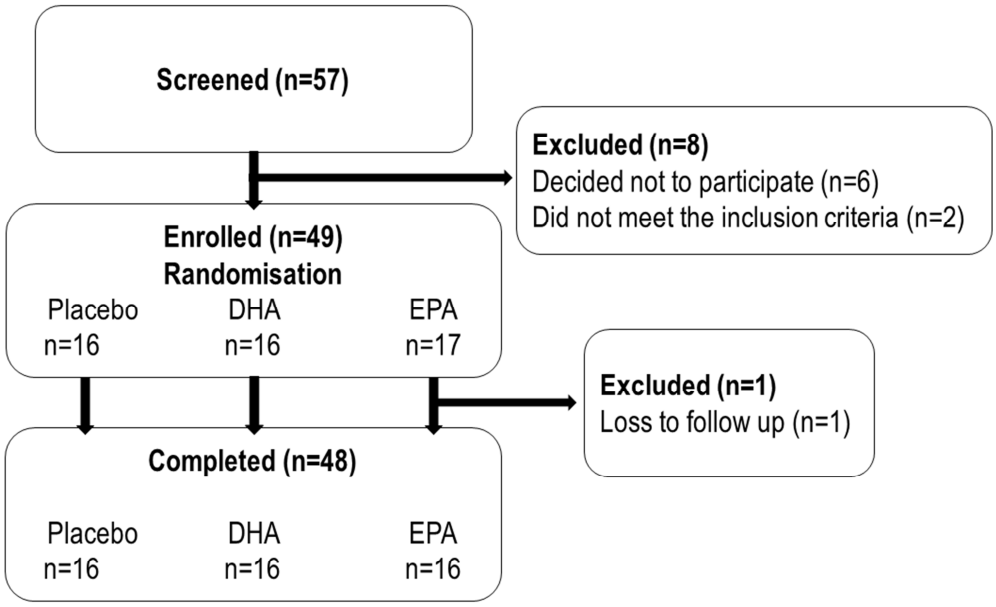
[1] geometric mean with $\log_e$ ratio of change from baseline

[2] *P* values are from univariate analysis of variances of the endpoint values by treatment with baseline values, ethnicity, BMI and age as covariates.

[a] significantly different from olive oil and DHA $P<0.05$, Bonferroni comparison test

FIGURE LEGENDS

Figure 1: CONSORT flow chart of participants in the EPA and DHA trial



Highlights

- We investigated the separate effects of EPA and DHA supplementation (3g/d, 6wks) on vascular and platelet function in healthy young males
- Neither EPA nor DHA influenced platelet monocyte aggregates or capillary density
- EPA, but not DHA, improved a marker of insulin sensitivity